DNAzyme-Directed Assembly of Gold Nanoparticles as Colorimetric Sensors for a Broad Range of Analytes

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***Abstract*—We have demonstrated the use of DNAzymes for directed assembly of gold nanoparticles in which the assembly state is highly dependent on the presence of specific analytes such as Pb^{2+} or adenosine. The resulting system has been used as highly sensitive and selective colorimetric biosensors for metal ions and organic molecules. The methodology is generally applicable to almost any chosen analytes and thus significantly expands the practical impact of nanotechnology on other fields and technologies.**

Keywords- DNAzyme; biosensor; nanoparticle; colorimetric; lead detection

I. Introduction

An important measure of success of nanoscale science and technology is its impact on other scientific fields and on everyday life. Recent progress in DNA-directed assembly of inorganic nanoparticles has resulted in new programmable nanomaterials with promising applications in electronic industry and in biotechnology [1-4]. For example, gold nanoparticles have well characterized optical properties, which are dependent on both the particle size and inter-particle distances. The very high extinct coefficient (ε=2.4×10^8 $M^{-1}cm^{-1}$ at 520 nm for 13 nm diameter particles) of gold nanoparticles makes them an excellent reporter system for colorimetric detection. Therefore, the use of DNA-functionalized gold nanoparticles for selective colorimetric detection of complementary DNA has been demonstrated and is playing an increasingly important role in genomic research and clinical tests [3,4]. The next challenge is to expand the technology beyond DNA detection to a broad range of analytes including metal ions, organic molecules, proteins, toxins and cells. The resulting colorimetric sensors would find significant applications in fields such as environmental and industrial process monitoring, developmental biology, and clinical toxicology. They may also play a role in anti- chemical and biological terrorisms.

Long considered as strictly a genetic material, it has been discovered that DNA/RNA can catalyze many chemical reactions as protein enzymes do [5]. Catalytically active DNA/RNA (called DNA/RNAzymes) can be obtained through combinatorial biology approaches such as systematic evolution of ligands by exponential enrichment (SELEX) or in vitro selection [6]. Similar to immunology, DNA or RNA that bind specifically to a broad range of analytes can be isolated from large libraries (often up to 10^{15} different sequences) through an iterative process of selection, amplification and mutation. A number of studies have now shown that the selected DNA/RNAzymes can often match antibodies in the broad range of analytes they can recognize (see Table 1) [7]. Several features make them excellent platforms for making sensors [8]. First, the selection is *in vitro* and thus less time- and fund-consuming. Second, unlike antibodies, DNA/RNAzymes can be denatured and renatured many times without losing the binding ability or activity, and can be used and stored under rather harsh conditions. Finally, the sensors can often be designed with minimum knowledge of the three-dimensional structures of the DNA/RNA molecules.

While a number of DNA/RNAzymes have been obtained that recognize a broad range of analytes, the next challenge is to transform the recognition into physically detectable signals. Toward this goal, conductivity, radioactivity, or fluorescence labeling methods have been developed [9, 10]. In many sensor applications, colorimetric detection can be advantageous because it can avoid difficulties associated with handling and disposing of radioisotopes, and eliminate or minimize most costs associated with instrumentation and operation in fluorescence detection and thus can make on-site, real-time detection easier [3].

TABLE 1. Analytes Recognized By DNA/RNA

Analyte type	Examples
Metal ions	Mg(II), Ca(II), Pb(II), Zn(II), Cu(II), Co(II)
Organic dyes	Cibacron blue, reactive green 19
Amino acids	L-Valine, D-Tryptophan
Nucleosides/nucleotides	Guanosine, ATP
Nucleotide analogs	8-oxo-dG, 7-Me-guanosine
Biological cofactors	NAD, FMN, porphyrins, Vitamin B_{12}
Aminoglycosides	Tobramycin, Neomycin
Antibiotics	Streptomycin, Viomycin
Peptides	Rev peptide
Enzymes	Human Thrombin, HIV Rev Transcriptase
Growth cofactors	Karatinocyte GF, Basic fibroblast GF
Antibodies	human IgE
Gene regulatory factors	elongation factor Tu
Cell adhesion molecules	human CD4, selectin
Intact viral particles	Rous sarcoma virus, Anthrax spores

Herein, we take advantage of the two emerging technologies of target specific DNAzymes and gold

nanoparticle-based detection and report the design of new DNAzymes for directed assembly of functionalized nanomaterials that can be used as simple colorimetric sensors for on-site and real-time detection and quantification of a wide variety of analytes including heavy metal ions, such as Pb^{2+} and organic molecules, such as adenosine.

II. Results And Discussion

A. *From Combinatorial Biology to Analyte-Specific DNAzymes*

A DNAzyme that is highly specific for Pb^{2+} has been obtained using an in vitro selection method mentioned in the introduction (Fig. 1) [11, 12]. In addition, we have also designed a "negative selection" strategy to improve the selectivity of the resulting DNAzymes [13].

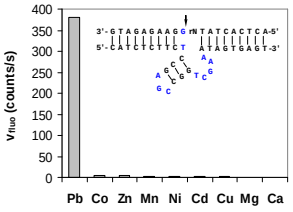

Figure 1. A highly Pb^{2+} selective DNAzyme

B. *Design of a Colorimetric Pb^{2+} Biosensor*

The selected DNAzyme is composed of an enzyme strand and a substrate strand (Figure 2a). The enzyme strand (in green) can catalyze the cleavage of the substrate strand (in black) in the presence of Pb^{2+} (Fig. 2b) [11, 12]. The cleavage site is indicated by a red color. This DNAzyme has very high Pb^{2+} specificity, which makes it possible to design biosensors for Pb^{2+}. Gold nanoparticles (13 nm diameter) were functionalized with 12mer thio-modified DNA. The substrate strand of the DNAzyme is extended on both the 3´- and the 5´-end for 12 bases, which are complementary to the 12mer DNA on the gold nanoparticles. Thus, the nanoparticles can aggregate with the substrate strand and the enzyme strand as linkers. The nanoparticle aggregate has a blue color. This blue aggregate can be used as a biosensor for Pb^{2+} [14]. If the aggregate is heated to ~50 °C and subsequently cooled to room temperature in the presence of Pb^{2+}, the substrate strand is cleaved by the enzyme strand and the re-formation of the blue aggregate is inhibited; a red color of individual gold nanoparticle is observed. In the absence of Pb^{2+}, the aggregate can be re-formed and a blue color is observed.

C. *Performance of the Colorimetric Pb^{2+} Biosensor*

The color change of the sensor induced by Pb^{2+} can be monitored by UV-vis spectroscopy. Typical UV-vis spectra of the DNAzyme-gold nanoparticle sensor in the absence and presence of 5 μM Pb^{2+} is shown in Fig. 3a. Upon aggregation, the extinction at 522 nm decreases, while the extinction at 700 nm increases. Thus, the ratio of the extinction at 522 nm and 700 nm is used to assay the degree of aggregation. A lower ratio means a higher degree of aggregation. This un-optimized sensor can detect Pb^{2+} from 0.1 to 4 μM (Fig 3c, solid squares).

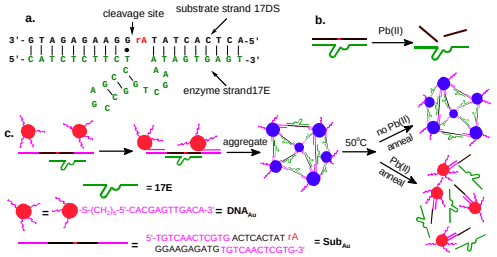

Figure 2. (a) Secondary structure of the DNAzyme system that consists of an enzyme strand (17E) and a substrate strand (17DS). The cleavage site is indicated by a black arrow. (b) Cleavage of 17DS by 17E in the presence of Pb^{2+}. (c) Schematics of DNAzyme-directed assembly of gold nanoparticles and their application as biosensors for Pb^{2+}.

D. *Colorimetric Biosensors with Tunable Detection Range*

By replacing a fraction of the active enzyme 17E with an inactive enzyme 17Ec, the detection range can be tuned over several orders of magnitude (Fig. 3c). 17Ec differs from 17E only by one base, but the activity is lost completely (Fig. 3b) [15]. When the enzyme strand is the active 17E only, the Pb(II) detection range is from 0.1 μM to 4 μM (solid green squares). When the ratio of 17E and 17Ec is 1:20, the Pb(II) detection range is from 10 μM to 200 μM (open green squares).

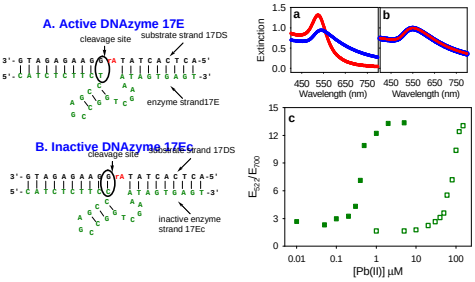

Figure 3. UV-vis extinction spectra of an active DNAzyme-nanoparticle sensor (a) and an inactive 17Ec DNAzyme-nanoparticle sensor (b) in the absence (blue curve) or in the presence (red curve) of 5 μM Pb^{2+}. (A) and (B) are active and inactive DNAzymes. (c) Tunable Pb^{2+} detection level.

E. *Colorimetric Detection*

The color can be conveniently monitored by spotting the sensor solution on a TLC plate. As the concentration of Pb^{2+} increases from 0 to 5 μM, a color progression from blue to purple to red can be observed (Fig. 4a), while all other competing metal ions at 5 μM concentration gives a blue color (Fig. 4b). We also tested the ability of the sensor to detect Pb^{2+} in paint (Fig. 4c) and shown that the sensor is quite sensitive in detecting and quantifying lead in leaded paint around 0.5%, the federal threshold for leaded paint.

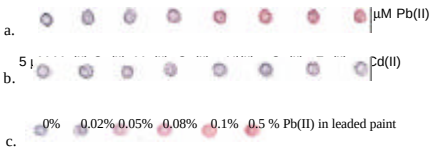

Figure 4. The color of the sensor developed on an alumina TLC plate with different Pb^{2+} concentrations (a) and with 5 μM of 8 other divalent metal ions (b). (c) Pb^{2+} detection in leaded paint.

F. Beyond Metal Ion Detection: A Colorimetric Sensor for Adenosine

While the above method has expanded the analyte range of DNA-functionalized gold nanoparticles system to many analytes beyond nucleic acids, it is still restricted only to those analytes (e.g., metal ions) that are directly involved in cleaving phosphodiester bonds. Therefore it is desirable to expand the above methodology to more analytes, regardless whether they can cleave phosphodiester bonds or not. Toward this goal, we have now designed a colorimetric adenosine biosensor based on the aptazyme-directed assembly of gold nanoparticles. The aptazyme is based on the DNAzyme with an adenosine aptamer motif that can modulate the DNAzyme activity allosterically depending on the presence of adenosine. In the absence of adenosine, the aptazyme is inactive and the substrate strands can serve as linkers to assemble DNA-functionalized gold nanoparticles, resulting in a blue color. However, the presence of adenosine activates the aptazyme, which cleaves the substrate strand, disrupting the formation of nanoparticle aggregates. A red color of separated gold nanoparticles is observed.

Fig. 5 shows the quantitative detection of adenosine using the method. Concentrations of adenosine of up to 1 mM can be measured semi-quantitatively by the degree of blue to red color changes, or quantitatively by the extinction ratio at 520 and 700 nm. Under the same conditions, 5 mM of guanosine, cytidine or uridine resulted in a blue color only, indicating excellent selectivity of the sensor.

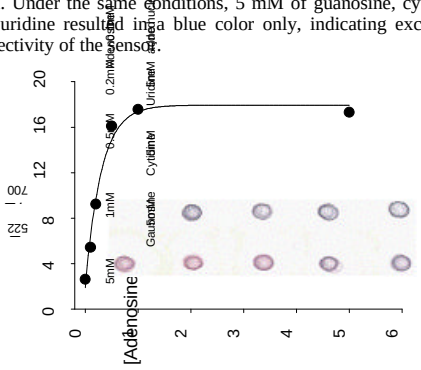

Figure 5. (a) Quantification of the adenosine concentration using UV-vis spectroscopy. (b) Detection by dropping the sensor on an alumina TLC plate.

III Conclusion And Outlook

We have demonstrated the DNAzyme-directed assembly of gold nanoparticles and its application as highly sensitive and selective colorimetric biosensors for metal ions such as Pb^{2+} and organic molecules such as adenosine. The methodology is general enough so that it can be applied to any chosen analyte to which the corresponding DNA can be obtained (see Table 1) and thus will significantly expand the practical impact of nanotechnology on other fields and technologies. At the same time, introducing highly specific biomolecules such as DNAzymes will allow us to transform nanomaterials syntheses from *self*-assembly to analyte-specific *directed* assembly, and thus can advance the nanotechnology field as well.

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